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Genotypes and Clinical Phenotypes of Hepatitis B Virus in Patients with Chronic Hepatitis B Virus Infection

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Genotype C of hepatitis B virus (HBV) has been shown to be associated with a poor clinical outcome, compared to genotype B. To explore the clinical phenotypes, with special reference to the seroconversion of hepatitis B e antigen (HBeAg) and frequency of acute exacerbation between patients infected with HBV genotypes B and C, a cohort of 272 Taiwanese patients with chronic HBV infection was analyzed. According to the status of HBeAg at enrollment and frequency of acute exacerbation during the follow-up period, five groups of patients with distinct clinical phenotypes were categorized. Of the 272 HBV carriers, 185 (68%) were infected with HBV genotype B and the remaining 87 (32%) were infected with genotype C. Among them, 150 (55%) were positive for HBeAg and patients with genotype C infection tended to have a higher positive rate of HBeAg than those with genotype B infection (63 versus 51%). Genotype B was more prevalent than genotype C in different groups of HBV carriers. However, the prevalence of genotype C in patients with multiple episodes of acute exacerbation who failed to have HBeAg seroconversion was significantly higher than in all 272 patients (50 versus 32%, $P = 0.025$), in those with HBeAg seroconversion after only one episode of acute exacerbation (50 versus 12%, $P = 0.01$), or in those negative for HBeAg at enrollment and without acute exacerbations (50 versus 23%, $P = 0.002$). In conclusion, patients with genotype C infection have a more aggressive clinical phenotype than do those with genotype B infection, which contributes to the former group's progressive liver disease and poor clinical outcomes.

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people are chronic carriers of the virus (3). The infection is associated with a wide spectrum of clinical manifestations, ranging from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carrier, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (1, 3). Although serological and genotypic classifications of HBV have been well documented (11), the clinical significance of HBV genotypes in terms of clinical outcomes and therapeutic response to antiviral therapy in patients with chronic HBV infection remains largely unknown until recently. Several studies suggested that HBV genotype C is associated with severer liver disease and a lower response rate to alpha interferon therapy than is genotype B (5, 6, 9, 13). Taken together, these data suggest the possible pathogenic and therapeutic differences among HBV genotypes. However, the pathogenic link between genotype C and the progression of liver disease remains unclear. We therefore studied the clinical phenotypes, with special reference to the seroconversion of hepatitis B e antigen (HBeAg) and frequency of acute exacerbation, for patients infected with HBV genotype B and genotype C.

MATERIALS AND METHODS

Patients. Between 1990 and 1995, a cohort of 272 patients (198 men and 74 women; ages, 16 to 70 years) with chronic HBV infection was followed at the gastroenterological clinic of National Taiwan University Hospital. Chronic HBV infection was defined as a persistent seropositivity for HBV surface antigen (HBsAg) for at least 6 months before enrollment. All patients were negative for antibodies to hepatitis C virus (anti-HCV) and hepatitis D virus and had no serological markers suggestive of autoimmune disease. None had a history of alcohol abuse (>50 g/day), parenteral drug use, or hepatotoxin exposure. No specific antivirals or immune modulators were given in the study period. These patients received liver tests including serum alanine aminotransferase (ALT) activity every 3 months, more frequently if indicated. Serial serum samples taken from each patient were stored at −20°C until use.

According to the status of HBeAg at enrollment and frequency of acute exacerbation during the follow-up period, five groups of patients with distinct clinical phenotypes were categorized. Group I consisted of 63 patients who had positive HBeAg at enrollment but without acute exacerbations or seroconversion of HBeAg during the entire follow-up period. Group II consisted of 41 patients who were positive for HBeAg at enrollment and had episode(s) of acute exacerbations before seroconversion of HBeAg. Among them, 16 had only one episode of acute exacerbation (group II-1) and the remaining 25 had multiple (≥2) episodes of acute exacerbation before seroconversion of HBeAg (group II-2). Group III consisted of 46 patients who were positive for HBeAg at enrollment and had multiple episodes of acute exacerbation but without seroconversion of HBeAg at the end of follow-up. Group IV consisted of 23 patients who were negative for HBeAg at enrollment and had episode(s) of acute exacerbation during the follow-up period. Group V consisted of 99 patients who were negative for HBeAg at enrollment and did not manifest acute exacerbations during their follow-up period. The positive rates of anti-HBe in group IV and group V patients were 86 and 95%, respectively. Acute exacerbation was defined as an abrupt increase of serum ALT (upper limit of normal, 40 U/liter) to a level greater than five times the upper limit of normal (200 U/liter) as previously described (4).

Hepatitis virus markers. Serum HBsAg and HBeAg were tested by Ausria-II and IMx HBe 2.0 (Abbott Laboratories, North Chicago, Ill.), respectively. Anti-HCV and anti-hepatitis D virus were tested by commercially available assays (HCV EIA II and Anti-Delta; Abbott Laboratories).

Genotyping of HBV. HBV genotypes were determined by using PCR-restriction fragment length polymorphism of the surface gene of HBV as previously described (5, 9). Six genotypes (A to F) of HBV could be identified by the restriction patterns of DNA fragments. To avoid false-positive results, instructions to prevent cross-contaminations were strictly followed, and results were considered valid only when they were obtained in duplicate. The sensitivity of our

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TABLE 1. Demographic and clinical data of 272 chronic hepatitis B patients with distinct clinical phenotypes

Group	No.	Sex (male/female)	Age (yr)	Mean peak ALT level (U/liter)
I	63	43/20	36 ± 10	129 ± 36
II	41	34/7	32 ± 7	490 ± 119
II-1	16	13/3	34 ± 8	236 ± 83
II-2	25	21/4	31 ± 7	651 ± 132
III	46	35/11	32 ± 8	529 ± 110
IV	23	14/9	39 ± 10	355 ± 140
V	99	72/27	45 ± 12	104 ± 34

PCR assay was 10 copies of HBV DNA per specimen by testing serial 10-fold dilutions of HBV DNA transcripts with known amounts (10^8 copies/ml).

Statistical analysis. Fisher's exact test, chi-square test with Yates' correction, and Student's *t* test were used where appropriate. A *P* value of <0.05 was considered statistically significant.

RESULTS

The demographic and clinical data of chronic hepatitis B patients with distinct clinical phenotypes are shown in Table 1. Patients in groups IV and V had a significantly higher mean age than those in other groups ($P < 0.01$ and < 0.001, respectively). Of the 272 HBsAg carriers, 185 (68%) were infected with HBV genotype B and the remaining 87 (32%) were infected with genotype C. Among them, 150 (55%) were positive for HBeAg at enrollment, and those with genotype C infection tended to have a higher positive rate of HBeAg than those with genotype B infection (63 versus 51%, $P = 0.1$).

The distribution of HBV genotypes in different groups of chronic hepatitis B patients with distinct clinical phenotypes during the entire follow-up period was analyzed (Table 2). In general, genotype B was more prevalent than genotype C in different groups of HBV carriers. However, the prevalence of genotype C in patients with multiple episodes of acute exacerbation who failed to have HBeAg seroconversion (group III) was significantly higher than that in all 272 patients (50 versus 32%, $P = 0.025$) and those with HBeAg seroconversion after only one episode of acute exacerbation (group II-1) (50 versus 12%, $P = 0.01$). A similar situation was also found in those negative for HBeAg at enrollment and without acute exacerbations (group V) (50 versus 23%, $P = 0.002$).

DISCUSSION

In addition to the serological classification of HBV isolates into nine subtypes according to the antigenic determinants of their HBsAg (2), a genetic classification based on the comparison of complete genomes has defined seven genotypes of HBV (A to G) recently (11, 15). Like HBV serological subtypes, HBV genotypes also have distinct geographical distributions (12). In general, genotypes B and C are prevalent in Asia, whereas genotypes A and D prevail in Western countries. Genotype E is restricted to Africa, while genotype F is found in Central America. Genotype G has been identified in France and North America very recently (15). Our results indicated that 68% of the Taiwanese hepatitis B carriers were infected with genotype B, confirming that genotype B is the most predominant HBV genotype in Taiwan (5, 7).

The clinical, virological, and therapeutic implications of HBV genotypes in patients with chronic HBV infection have been partially clarified. Although several studies have suggested that HBV genotype C is associated with the severity of liver disease (4, 5, 9, 13), the pathogenic link between genotype C and the progression of liver disease remains largely unknown. In the natural course of chronic HBV infection, early seroconversion from HBeAg to anti-HBe (immune clearance phase) usually indicates a favorable outcome, because it is usually associated with the cessation of virus replication and nonprogressive liver disease (1). In contrast, late seroconversion of HBeAg after multiple episodes of reactivation and remission may accelerate the progression of chronic hepatitis B and thus have a poor clinical outcome (14). Our previous data implied that genotype C seems to stay longer in the immune clearance phase of persistent HBV infection and shifted to stages of severer liver inflammation, and genotype B may be associated with a faster transition through the immunoreactive stage and evolve into the residual phase in which the serum HBV DNA becomes barely detectable (5). Although not statistically significant, our results suggested that patients with genotype C infection tended to have a higher frequency of HBeAg positivity than those with genotype B infection (63 versus 51%, $P = 0.1$). In addition, when the seroconversion of HBeAg and frequency of acute exacerbation were used to define the clinical phenotypes of patients with chronic HBV infection, our data showed that the prevalence of genotype C in HBV carriers with multiple episodes of acute exacerbation who failed to have HBeAg seroconversion was significantly higher than in all 272 patients (50 versus 32%, $P = 0.025$ [Table 2]), in those with HBeAg seroconversion after only one episode of acute exacerbation (50 versus 12%, $P = 0.01$ [Table 2]), or in those negative for HBeAg at enrollment and without acute exacerbations (50 versus 23%, $P = 0.002$ [Table 2]). These findings suggested that genotype C is associated with persistently HBeAg-positive chronic HBV infection with multiple episodes of acute exacerbation and further supported our prior observations (5). Previous studies also indicated that the frequency and severity of acute exacerbation of chronic hepatitis B are closely associated with the development of liver cirrhosis in HBsAg carriers (8, 10). In the meantime, double mutations of the core promoter have been claimed to be associated with increased viral replication, active liver histology,

TABLE 2. Distribution of HBV genotypes B and C in different groups of chronic hepatitis B patients with distinct clinical phenotypes

Group	No.	No. (%) of patients	
		Genotype B	Genotype C
I	63	40 (63%)	23 (37%)
II	41	32 (78%)	9 (22%)
II-1	16	14 (88%)	2 (12%)
II-2	25	18 (72%)	7 (28%)
III	46	23 (50%)	23 (50%)[b]
IV	23	14 (61%)	9 (39%)
V	99	76 (77%)	23 (23%)
Total	272	185 (68%)	87 (32%)[a]

[a] $P = 0.025$ versus group III. *P* was not statistically significant versus groups I, II, II-1, II-2, IV, and V.

[b] $P = 0.01$ versus groups II and II-1. $P = 0.002$ versus group V. *P* was not statistically significant versus groups I, II-2, and IV.

and more advanced liver disease (6, 9, 13, 14), and such mutations are frequently found in genotype C strains. Taken together, it is therefore reasonable to speculate that the higher frequency of HBeAg positivity, the longer stay in immune clearance phase, and the more frequent core promoter mutation of HBV genotype C may contribute to the poor clinical outcomes of patients with genotype C infection compared to those of patients with genotype B infection.

In summary, our results suggest that patients with genotype C infection have a more aggressive clinical phenotype than do those with genotype B infection, which contributes to genotype C patients' progressive liver disease and poor clinical outcomes.

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